On the Structure and Development of the Ovaries and their Appendages in the Blowfly (*Calliphora erythrocephala*). By B. Thompson Lowne, F.R.C.S., F.L.S., Hunterian Professor of Comparative Anatomy in the Royal College of Surgeons.

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(Plate XXVIII.)

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1. *Introductory.*

The ovary of an insect is known to consist of a number of ovarian tubes connected with a single outlet, the oviduct.

The ova lie within a thin membranous tube, the *tunica propria*, and form an egg-string; this is enclosed within a follicle, the egg-follicle, the so-called peritoneal coat. In each egg-string three parts are usually recognized—the terminal thread, the terminal chamber, and the egg-chamber or chambers.

In the Blowfly each ovary consists of about a hundred ovarian follicles, springing from the dilated anterior end, or calyx, of the ovarian duct. When the ovary is mature (Pl. XXVIII. fig. 8) the terminal threads exist as mere rudiments (*t.f.*), and, properly speaking, there are no terminal chambers. In the young ovary, however (fig. 6), the terminal chambers are well marked (*t.c.*).

Much discussion is found in the writings of various authors as to the nature and import of the terminal thread.

Brandt (6*) maintains that there is no fundamental difference between ovaries with and ovaries without terminal threads (*l. c.* p. 21)—"these are mere prolongations of the ovarian tubes or of their peritoneal investment. In the former case they have the same morphological significance as the rest of the ovarian tube, in the latter they are mere connective or suspensory bands." My own observations have led me to exactly the same conclusion.

The Terminal Chamber (fig. 6, *t.c.*) is filled with small cells. Stein (24) was apparently the first to distinguish the terminal chamber as an important constituent of the ovary; he calls it the

* The numbers in parentheses following authors' names refer to corresponding numbers in the Bibliography given at the end of this paper.

germinal chamber (*Keimfach*). He was followed by Prof. Huxley (10, 11), Sir John Lubbock (19), Claus (8), and others. Stein enunciated the view that the function of the terminal chamber is the formation of germ-yelks; but he does not regard all the cells in the chamber as germ-yelks. Sir John Lubbock went a step further and wrote as follows:—"In their earliest stage, the egg-cell and the vitelligenous cells cannot be distinguished from each other, and no one, I think, who has carefully examined the upper part of the egg-tube in any Hemipterous or Dipterous insect can fail to be of the same opinion."

I agree entirely with Sir John Lubbock in this, that all the cells in the terminal chamber are alike; but when he concludes, "The egg-tube contains, indeed, at this end, cells which are neither vitelligenous nor egg-cells, but which are capable of becoming, under certain circumstances, either one or the other," I cannot agree with him, and my reasons will appear in the sequel.

The Egg-chambers.—This term was first applied, I believe, by Brandt (6) to that portion of the egg-tube which contains definite ova. In some insects each egg is formed from a single cell; this is so in the Orthoptera; such ova are designated by Brandt *panoïstic.* In other insects several cells are concerned in the formation of the ovum; these ova he termed *meroïstic.*

In the meroïstic egg Brandt calls the lowest cell the egg-cell, the others he terms nutrient or yelk-cells.

The part played by these nutrient cells is a matter upon which there is great divergence of opinion. Brandt's view, which has been generally adopted in text-books and widely accepted, is this:—The egg-cell in the meroïstic egg is the only cell enclosed by the chorion, and the nutrient cells remain outside and ultimately disappear. These are supposed to be in some way concerned in the nutrition of the egg-cell. The great increase in the size of the egg-cell is due to the deposition of yelk-granules within it, around its nucleus, which Brandt regards as the germinal vesicle. Similar changes also occur in the panoïstic egg, which only differs from the meroïstic in the absence of the nutrient cells.

Weismann (26) maintains, on the other hand, that all the cells, the nutrient as well as the egg-cell, are enclosed in the chorion, and that they all take part in the formation of the yelk, ultimately fusing into a single mass; and this, as I shall show hereafter, agrees with my own observations.

With regard to the import of Brandt's egg-cell there is less divergence of opinion; the identification of its nucleus with the germinal vesicle has been regarded as of the highest importance, and numerous attempts have been made to show that the nucleus of the egg-cell differs from those of the nutrient cells, from the very earliest stages of the egg-formation. Thus Dr. Claus (8) (p. 44) writes:—"The questions, the answers to which are of the highest importance, are: from whence is the germinal vesicle derived? and what is its relation to the great yelk-cells? The identification of the germinal vesicle appears to be difficult, and the earlier observers came to no certain result." After quoting from Sir John Lubbock's paper (19) he continues, "I believe my own observations enable me to prove that the epithelial cells, the yelk-forming cells, and the egg-cell are modifications of originally identical elements." Yet Claus thinks he distinguishes the germinal vesicle in very immature ova by "its smaller size and clearer contents" from the nuclei of the adjacent yelk-cells. Meyer's (20) statements are in direct opposition to this; according to these there are several germinal vesicles, each nucleus becomes invested by a membrane, each making, as it were, an attempt at forming an egg, the lowest nucleus persisting and the others disappearing.

In my opinion by far the best and most accurate description of the development of the ovarian eggs in the Fly is due to Weismann (26); it is as follows:—"The ovaries are developed very slowly in the Muscidæ; when the fly emerges from the pupa none of the eggs are formed, although the ovary may be recognized in the embryo" (*l. c.* p. 206). Weismann then refers to a description of the ovary in the adult larva of the closely-allied *Sarcophaga carnaria* (at page 134), "Concerning the female sexual organs in the larva of *Musca vomitoria* I have no observations; I must therefore fall back on those on the closely-allied *S. carnaria*. In a larva one centimetre long, the ovary has a diameter of $0{\cdot}29^{m}$, is flask-shaped, and differs in its histological structure from the male sexual gland; here we find no mother-cells, the structureless sheath encloses only small round cells $\cdot013^{m}$ in diameter with nuclei $\cdot01^{m}$, exceedingly clear and exhibiting nucleoli. These cells are isolated with difficulty, as they adhere closely to each other. If we tear the ovarium of an adult larva no further structure is visible; but if a gentle pressure be applied to the uninjured ovary one may distinguish the first rudiments of the ovarian tubes. In the upper half of the ovary they appear as cylindrical follicles lying parallel to each other. They are all united above without any point (terminal thread ?) being visible, below they are lost in the cellular mass. The diameter of a follicle averages ·04 m. The ovarian follicles consist of a sheath of fine structureless membrane and its contents, which differs in no way from the surrounding cell-mass. The sheaths are a cuticular excretion from the outer surface of the cells forming the cylinders." (He continues on page 206) "So that, as I have shown above, the original soft mass of cells with which the ovarian capsule is filled becomes differentiated, in part, into solid strings, which shed a cuticle from their surface, and the ovary comes to consist of a small-celled ground-substance which fills the capsule, in which solid cellular strings are imbedded, each enclosed in a fine structureless membrane; of an outer and inner epithelium, a tender albuminous contents in which free nuclei are imbedded, as Meyer describes in the youngest condition of the ovary, there is as yet no trace. The term egg-tubes is hardly admissible at this stage, it is only later by the differentiation into a wall and contents that they become tubes."

"On the seventh day of the pupa stage the egg-tubes still only occupy a small zone of the flask-shaped ovarium (Taf. xiv. fig. 70); they lie close together parallel to the long axis of the ovary and still exhibit their original simple structure, only the contained cells are somewhat larger and therefore more distinctly seen. These cells are spherical, and their nuclei are easily distinguished. The cuticular sheaths end above in rounded domes."

"By the fourteenth day the investing sheaths of the egg-tubes are considerably more developed, and their outer form is altered; the blind end is now drawn out into a point, the middle part is swollen and the posterior part contracted. Still the lumen is filled with cells disposed without definite order; no regular epithelium is yet visible, but there is a great difference in the size of the cells, the central ones being larger than those of the periphery. A little later these changes are more conspicuous, and the egg-tube exhibits a stem, a chamber, and a nipple-like appendage" [Stein's end-chamber], "the narrowed blind end of the tube. In the chamber there is a distinct separation of the cells, small cells line the follicle in a single layer, as an epithelium enclosing the larger cells; from the latter the egg is ultimately formed."

"The development of the ovary shows that the life of the fly must last several weeks. A ripe egg is first found in the lowest part of the ovarian follicle after the insect has flown about for a long time; then a second, third, or even a fourth chamber has been developed in which there are eggs in different stages of formation."

"The development of these eggs takes place as follows. The large cells which lie within the epithelium of the egg-chamber enlarge, by their rapid growth they lose their original spherical form and appear flattened against each other as more or less hexagonal sections of a sphere."

"These cells each enclose a very distinct transparent vesicular nucleus, and consist of homogeneous, but highly refractive cell-substance. With increase of the cells by growth this cell-substance becomes finely granular and afterwards dark and yelk-like. The cell-membranes then disappear, and the yelk formed in the cells fuses into a mass; so also all the nuclei disappear except one, which becomes the germinal vesicle. It appears that the nucleus of the cell which lies lowest in the chamber always furnishes the germinal vesicle. This seems to have orginated Meyer's statements."

Weismann concludes with the words *, "So far as the Diptera are concerned, my view accords with Lubbock's; we agree that the egg of the Diptera is not derived from a single cell, but is a compound formation, like the egg of Cestodes or Trematodes, in which a germogen and vitelligen combine their products, for the composition of an egg."

Stuhlmann (25) holds the same views as Brandt with regard to the fate of the nutrient cells, and renews the old controversy with regard to the germinal vesicle. The principal results at which he arrives concerning it are summed up by him in the following words:—"I have been enabled by a series of observations on insects' eggs to establish the extrusion of large balls from the germinal vesicle which are afterwards lost in the egg-plasm. Later the germinal vesicle disappears until at last at the upper egg-pole we again find it as the segmentation nucleus" †.

* Dass das Ei der Dipteren nicht von einer einzigen Zelle abstammt, sondern ein ebenso zusammengesetztes Gebilde ist als die Eier der Cestoden und Trematoden, bei denen Dotterstock und Keimstock ihre Producte zur Bildung des Eies zusammenfliessen lassen" (*l. c.* p. 209).

† "Es ist mir nun gelungen, an einer Reihe von Insekteneiern sicher einen Austritt von grossen Ballen aus dem Keimbläschen zu constatiren, die sich

Such an outstreaming of nuclear particles (*Ballen*) is undoubtedly seen in the lowest nucleus of the egg, but it also occurs in the nuclei of the so-called nutrient cells, and in all the nuclei of the various organs of the larva during their degeneration in the first stages of the pupa. It is one of the most characteristic phenomena of yelk-formation, whether in the egg or the pupa, whilst it is quite unlike anything which has been observed in relation to the well-known germinal vesicles of other animals.

Lastly, Henking (9) has quite recently figured and described the ripe ovarian egg of the Blowfly with the nutrient cells outside the chorion, and his figure has fortunately enabled me to discover the error into which Brandt and his followers have fallen. The appearance represented by Henking is an exceptional phenomenon which I have frequently observed. When the eggs approach maturity they enlarge so rapidly that the anterior pole of an egg is often pushed into a chamber above it containing a half-developed ovum, which then assumes the form of a cap over the anterior pole of the ripe egg. I have sections which exhibit this phenomenon in several stages. Whenever the young ovum in the chamber above the ripe egg is present in an un-deformed condition the cap on the ripe egg is absent, and whenever a cap is present there is no second chamber in the egg-follicle. So many of the egg-tubes exhibit transitional conditions in which the ripe egg impinges upon or slightly indents the half-formed egg in the chamber above it that, with good sections, I cannot believe anyone would have the slightest doubt as to the nature of the phenomenon.

2. *The Development of the Ovaries and Ova.*

The earliest stage of development in which I have as yet seen the ovaries of the Fly is in the four-day old pupa (Pl. XXVIII. fig. 4). In this stage they are apparently slightly in advance of the stage described by Weismann as that of the seven-day-old pupa. The discrepancy is probably due to the fact that I worked in summer, and Weismann's observations were made in winter. The earlier stages of the pupa are well known to be greatly influenced by the external temperature.

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nachher im Eiplasma auflösen. Später verschwindet das Keimbläschen von unseren Blicken, bis wir endlich am oberen Eipol den Furchungskern wiederfinden" (*l. c.* p. 12).

At this stage the ovary is pear-shaped ·25 m. in diameter and ·34 to ·4 m. in length. It is enclosed in a thin but perfectly distinct cellular capsule (*c*). It consists of a stroma of small cells less than 5 μ in diameter, enclosing the bundle of egg-strings (*e.s*). These are closely packed together and occupy the anterior rounded half of the ovary.

The posterior narrow part of the ovary (*cl*) is filled with small round cells precisely like those which form the egg-strings, except that the latter are slightly larger, 5 μ.

The egg-strings present, even at this period, a narrower constricted posterior and a more dilated anterior portion; they are like long narrow flasks, the neck measures 5 μ and the dilated portion 15 μ in diameter. The whole consists of small closely packed cells enclosed in a fine cuticular membrane. The necks of the egg-strings appear to be open behind, where the cells of the posterior part of the ovary, destined to form the duct, are continuous with those within the flask-like egg-strings. There is as yet no trace of a lumen in the solid rudiment of the oviduct.

The cells between and around the egg-tubes are, however, already distinctly elongated and form a stroma, in which the egg-strings lie, so differing entirely from the cells which form the egg-strings and fill the calyx of the ovary.

The next stage of development is seen in the half-formed pupa, about the tenth day (Plate XXVIII. fig. 5); at this stage the ovary is apparently cup-shaped, it appears crescentic in lateral sections, with the concavity of the crescent in front; it has a diameter of ·5 m., but is still about ·3 m. in thickness, measured from before backwards in its thickest part. That part of the ovary not occupied by the egg-strings is excavated by numerous channels; these form the calyx of the oviduct and cover the whole convex surface of the organ. The egg-strings are now so broad in front that I shall term them egg-follicles. The egg-follicles (*os.*) are ovoid masses of small cells, each with a very narrow stalk (*st.*) The stalk is apparently filled by a single row of cells, and its investing cuticle is frequently transversely wrinkled, which often produces an appearance of striation. Possibly this may have given rise to the very remarkable view held by Schneider (23) that the egg-tubes are developed within the alar muscles of the dorsal vessel.

Each rudimentary egg-follicle is now surrounded by a pouch, the ovarian follicle (*of.*), formed of fusiform mesoblastic elements. This is the so-called peritoneal coat of Stein. The ovarian follicles at this stage do not appear to open directly into the channels in the calyx of the ovary, but to be closed by a cellular mass (x), with which the stalks of the egg-follicles are continuous. This cellular mass appears to me to be formed by the cells which filled the posterior part of the ovarian capsule at the earlier stage of development above described.

Between the ovarian follicles, which no longer lie close together, the elements of the pseudo-yelk of the pupa (*psy*) are found in abundance. These consist of globules (*Kornchenkugeln*) and leucocytes; they are derived from the breaking up of the fat-bodies and the tissues of the larva. The pseudo-yelk, at this period, forms the greater part of the bulk of the pupa. Indeed, if such a pupa be opened it appears to contain nothing but a milky fluid, in which all the tender half-developed imaginal tissues are concealed.

There is as yet no differentiation of the contents of the egg-follicles into epithelium, yelk, or germ-cells.

Even at the time when the fly is ready to escape from the pupa the ovary remains in a condition which differs but little from the stage last described, except in the form of the egg-follicles, which now exhibit a narrow stalk and an ovoid middle portion constricted above so as to form a small, but distinct nipple-like terminal chamber (fig. 7, *t.c.*).

The peritoneal coat (*of*), or ovarian follicle, is also more developed, is much thicker, and exhibits numerous tracheal vessels (*tr.*). There is still, however, no trace of differentiation in the cells contained within the egg-chamber, and these only differ from those in the terminal chamber in being slightly larger.

In the observation of the further developmental changes we must have recourse to flies which have been on the wing for some time, and therefore the age of these insects is unknown. Development progresses very slowly in captive insects, and as these never unite with the males, there is no certainty that development progresses at the same rate or in the same manner as in free insects.

The next stage which I shall describe is represented in Pl. XXVIII. fig. 6. The ovary now measures ·35 m. in thickness and is discoid. The calyx is very thin, as the ducts which form it appear to be flattened by the lateral growth of the organ.

The whole ovary has also altered its position; the surface from which the oviduct originates is now turned towards the ventral aspect of the insect, so that the long axes of the egg-follicles are transverse to the axis of the body. I shall still, however, call the end of the egg-follicle which is nearest to the oviduct, posterior, to facilitate comparison and avoid confusion.

The ovarian follicles are now more fully developed, and loosely invest the posterior part of the egg-strings. The anterior part, the terminal chamber, is closely covered by the anterior part of the follicle, which is so thin that it can scarcely be traced as a distinct layer. The posterior part of the egg-follicle exhibits a distinct epithelial layer, which is continuous with the epithelium of the calicine duct, and the follicles open freely into these ducts.

The three parts of the egg-string are very distinct, within a very fine cuticular tunica propria. The stalk is filled by a single layer of epithelium; there is no lumen and no double epithelial layer; but where the stalk enlarges near the egg-chamber there is more than a single layer of cells, but these are irregularly arranged.

The egg-chamber, when fully formed, contains a group of yelk-cells flattened by mutual pressure and surrounded by an epithelial capsule (*ec*), which is continuous with the cells of the stalk.

In some of the tubes a second egg-chamber is seen in process of formation (Pl. XXVIII. fig. 7), with a cup-like epithelial investment. In others the first egg-chamber is in the same condition; the cells destined to form the egg are still round and scarcely differentiated from those of the terminal chamber. It appears to me that the epithelial investment of the ovum grows up from the stalk, and that all the cells of the terminal chamber develop into yelk-cells.

The gradual transition from the small round cells of the terminal chamber to yelk-cells is very marked, so also is the upper edge of the epithelial cup (fig. 7, *y*), which ends abruptly in a thin edge, whilst the continuity of the epithelium of the stalk and of the egg is equally distinct.

The large yelk-cells stain very deeply with alkaline carmine, and the colour is not washed out by 5 per cent. solution of acetic acid. They average 12 μ in diameter, and all the cells in an egg are precisely similar, and have nuclei which are rich in chromatin granules. There is no cell with a clear nucleus, and nothing which I can recognize as a germinal vesicle.

In mature insects ready to lay eggs the ovaries occupy the greater part of the cavity of the abdomen. There are about one hundred egg-tubes (80 to 100) in each ovary, each having four, five, or even six egg-chambers in different stages of development (fig. 8).

A mature egg occupies the most posterior chamber (fig. 8, *a*), a partially formed egg is seen in the second chamber, whilst the third, fourth, and terminal chambers contain very rudimentary ova. The whole terminates in a small empty, hollow, end-thread (*tf*).

The cuticular tunica propria closely surrounds the egg and egg-strings, whilst the thin greatly distended egg-follicle passes from one egg to the other, leaving a considerable space between the eggs; it does not follow the outline of the egg-string between successive ova. The egg-string between the second and third chambers (tp^1) is exactly similar to the egg-stalk of the first chamber in the early stages of its development.

The two or three anterior chambers are filled with small round cells like those of the terminal chamber or the whole egg-follicle at an earlier period. The unripe egg in the second chamber (ec^2) consists of large yelk-cells enclosed in an epithelial chorion. The lowest cell in such eggs is, however, always much larger, usually twice as large as the others, but its nucleus is also nearly twice as large, and stains just as deeply. It also contains the same kind of granules as the others. In these statements I agree in no way with Brandt and Stuhlmann. Brandt states that the nucleus of the lowest cell is large and clear, Stuhlmann that it is very much smaller than the other nuclei, and that it is clear and flattened against the chorion. According to my observations it is neither one nor the other, and only differs from the nuclei of the other cells in being larger.

The yelk-cells ultimately attain a giant size; the largest cell, when full-grown, measures 200 μ in its longest diameter, and has a nucleus of 80 μ in diameter. When the egg is enlarged to about two thirds of its maximum size the granules in the largest nucleus appear to stream out, the nucleus itself shrivels and is ultmately lost, whilst the whole protoplasm of the cell assumes a granular yelk-like appearance, in which the nuclear granules can no longer be distinguished. The remaining cells undergo the some changes, and soon become fused with each other and with the yelk formed from the lowest cell.

The nuclei during these changes present a very variable appearance; but all the changes of the nucleus are similar to those which characterize the nuclei of the degenerating cells of the larva, during the formation of the pseudo-yelk of the pupa—a phenomenon well seen in the nuclei of the cells of the salivary glands and fat-bodies of the larva during their histolysis.

I conclude therefore that the several cells from which the yelk of the Dipterous egg is formed are of equal morphological significance, that these all undergo histolytic changes, and so form the yelk of the mature ovarian ovum.

So far as my observations go, there is no reason for supposing one nucleus rather than another is the germinal vesicle.

When I first began this investigation, more than two years ago, I looked for days in vain for some character by which I might recognize the germinal vesicle. Sometimes one nucleus, sometimes another presents a clearer contents and smaller diameter, and frequently several nuclei appear to possess equal claims in this respect to be considered the nucleus of the germ-cell.

As the young ova approach the condition of maturity, the cell-substance becomes more and more distinctly granular, the nuclei lose their sharp contour, and exhibit what Stuhlmann describes as an extrusion or outstreaming of nuclear particles, whilst these are lost to view in the granular surrounding protoplasm, and the cells themselves become fused into a single yelk-mass. These changes commence in the lowest and largest cell of the egg; but precisely the same changes afterwards occur in the remaining cells as each attains its full growth.

The mature ova consist of a yelk surrounded by two membranes, the vitelline membrane and the chorion. Such ova are closely embraced by the structureless cuticular membrana propria, and lie loosely in the distended ovarian follicle, which is now a very thin-walled tube surrounded by a dense network of tracheal vessels.

The yelk consists of an outer clearer layer (Pl. XXVIII. fig. 11, *a*) and an inner granular substance (fig. 11, *b*), but neither contain any nuclei or cellular elements of any kind.

The clear peripheral layer of the yelk exists in the unimpregnated eggs whilst they still lie in the ovary; this layer was described by Weismann, and called by him the *Blastoderm plasma* (*Keimhautblastem*). He supposed that it is this layer which forms the blastoderm. In my sections it projects in places as if it possessed the power of amœboid movement, more especially at the anterior egg-pole * (fig. 10, *a*); these may, however, be the result of post-mortem contraction. The central granular yelk-substance consists of small granules, 2 to 3 μ in diameter (fig. 11), imbedded in an apparently structureless, possibly in the living egg semifluid, matrix. These granules are spheroidal, stain deeply, and exhibit either a dark or light centre with alterations of the focus of the microscope. In the ripe unimpregnated ovum I have entirely failed to find any nuclei or cellular elements of any kind, and I feel sure that if any such elements were present they would be readily distinguished in my sections.

It is not necessary for my purpose to enter into any details in regard to the structure of the chorion and the nature of the vitelline membrane; there are, however, some controverted points upon which I would say a few words.

It is generally held that the epithelium of the egg forms the chorion as an exudation from its inner surface (E. Korschelt, 14; Weismann, 26). Whether this is so or whether the chorion is formed from the cells themselves (Leuckart, 16), the manner in which the ova leave the oviducts is entirely in favour of the latter view. This is effected by the rupture of the remaining rudiment of the egg-string between the ripe egg and the imperfect ovum immediately in front of it. Thus the thin tunica propria and the epithelium of the egg descend in the ovarian follicle and enter the oviduct together. The remains of the egg-string attached to the unripe ovum in the ovarian follicle have been seen and described by several observers, notably by Müller, Landois (15), and Leuckart (16). I hold therefore that whether the cellular epithelium is shed with the thin cuticular egg-sheath in the oviduct, or whether it remains as the chorion

* The polar globules of Robin, which he described as formed by budding and fission, are possibly only mobile processes of this layer in a contracting yelk.

itself, it belongs entirely to the ovum, and cannot be regarded as the epithelium of the ovarian follicle, which is quite distinct and remains in the follicle.

I am also inclined to regard the vitelline membrane as the cuticular exudation from the inner surface of the epithelium of the ovum and the chorion as the modified epithelium itself. The cuticular sheath which leaves the ovarian follicle with the egg is, I have little doubt, the epichorionic membrane described by Leuckart, Robin, and Kölliker. The shedding of the outermost covering of the egg, probably the epichorionic membrane, and possibly also of the epithelial chorion, was observed by Brandt in the Field-crickets in transit through the oviduct, forming what he designates corpora lutea.

The micropyle-canal, which, in the Diptera, extends the whole length of the dorsal surface of the egg, is an infolding of the chorion (Pl. XXVIII. fig. 9). It is extended over the anterior egg-pole (fig. 10), forming a considerable chamber in the floor of which the micropyle is situated (fig. 10, *m*).

The micropyle (figs. 12 & 13) is a small, almost quadrilateral opening (fig. 12), 2·5 μ in diameter; it is surrounded by a number of radiating folds which project on the outer surface of the chorion, and by a circular area composed of small hexagonal cells. These correspond in size to the hexagonal fields with which the rest of the chorion is sculptured.

The open micropyle-canal is brought into relation first with the orifices of the gum-glands, and later with those of the spermatophorous capsules during the descent of the egg through the genital canal. Henking (9) found spermatic filaments in the micropyle-canal.

3. *The Oviducts and their Appendages.*

The general form and arrangement of these parts is well known, so that the following description will suffice to indicate their arrangement for my present purpose.

The ovarian ducts are two in number (fig. 2), and these form a common oviduct (*od*) by their union. The common oviduct opens into the pouch-like anterior extremity of the vagina on its dorsal aspect.

Stein describes it as opening on the ventral surface in Beetles. I formerly fell into the same error; and it is exceedingly difficult in dissections to determine this point. Sections of the entire insect show at once the true relations of the parts.

The pouch-like anterior part of the vagina is very distinct from the posterior part; that portion of it in front of the orifice of the common oviduct (fig. 3, *b*) in the young insect is the *bursa copulatrix* of authors. The *bursa* in the egg-laying insect is no longer distinct, but forms the anterior part of the vaginal pouch.

I shall call the vaginal pouch the uterus, a term applied to it by Palmén (21) to distinguish it from the posterior tubular part of the vagina. If the term is not morphologically, it is at least physiologically correct, as an egg is frequently retained in it until the embryo is ready to escape from the shell.

There is at present some uncertainty as to the exact manner in which the common oviduct is developed; although it is quite certain that the ovarian ducts are developed from the posterior portion of the ovary, as Palmén has distinctly shown (21). The same observer also shows that the vagina, uterus, and their appendages are formed by an invagination of the external integument, or rather of the hypoderm. My own observations entirely confirm Palmén's statements. Although I have not been able to trace the development of the common oviduct, its structure and the manner in which the common duct of the testicles is formed in the Fly (Weismann, *l. c.* Taf. xiv, fig. 68) indicate that it is formed from the prolonged posterior parts of the ovaries.

So far I have stated nothing concerning the anatomy of these parts which has not been frequently observed and generally admitted. I must now, however, enter into some details which are not, so far as I know, to be found elsewhere.

The common oviduct in the Blowfly terminates in two distinct enlargements (figs. 1 and 3). The more anterior is due to a thickening of its muscular coat where a thick retractor muscle (*m*) is inserted into it. This withdraws the parts with the ovipositor. The second or terminal enlargement (*os*) is, however, a pouch or bulb lined by a greatly plicated intima, and capable of distention, so that it encloses the entire egg during its passage through the oviduct. One egg is frequently found in this section of the oviduct whilst another occupies the uterine cavity.

The great interest of this pouch is that the gum-glands (*gl*) or colleterial glands, as they are sometimes called, open by two slender ducts (*d*) into it, and not, as is usually believed, into the uterus itself.

Although I have frequently satisfied myself of this, both by section and by careful dissection, this point is of such importance, that I shall enter into an examination of the views of previous writers with regard to the termination of these ducts.

It is quite possible that several distinct glands have been confounded under the term gum-glands; indeed it is generally used for any accessory gland connected with the sexual canal. These glands are generally described as opening into the vagina or uterus. Stein (24) gives a great number of figures representing the oviducts, uterus, and appendages in the Coleoptera; in many it is difficult to identify the gum-glands. In *Hydrophilus* (*l. c.* Taf. iv. fig. iii) he represents the gum-glands as opening into the upper part of the ovarian duct. They are branching tubules which evidently form part of the ovary itself; and, judging by his excellent figure, are identical with the so-called gum-glands in the Blowfly.

Except in the Hydrophylidæ, Stein considers the gum-glands as a portion of what he terms the "apparatus of fertilization" (*Befruchtungs-Organe*), and represents them as if they opened into the spermatophorous capsules or their duct; although in many cases it is almost evident from his figures that they open into the oviduct. In some of his figures the spermatophorous capsules are represented opening into the oviduct (Taf. i. fig. vi), whilst in others they are correctly represented opening into the uterus, whilst the gum-glands open into the oviduct (Taf. ii. figs. i, ii, and iii).

Tracing the gum-gland in the Blowfly from its ovarian extremity, it lies first under and close to the ovarian duct; it then leaves this duct and comes into relation with the spermatophorous capsule, around which it forms a loop. The duct of the gum-gland commences at the termination of this loop, and is easily overlooked, as it is in close contact with the duct of the spermatophorous capsule, round which it turns and runs forward in close contact with the dorsal wall of the uterus and oviduct to terminate in the bulb of the oviduct. It is not difficult to understand how this duct has been overlooked, or how it has been supposed that the glands open into or with the seminiferous capsules. The gum-glands have also probably been confounded with true vaginal glands, which appear to exist in some insects.

I shall again refer to the gum-glands in a special section of the present paper in relation to their structure and functions.

The uterus (figs. 1, 2, 3, *ut.*) is a thick-walled sac lined by a strong cuticular membrane, very different to the thin cuticular membrane lining the oviduct. It has a diverticulum or pouch (*p*) on its dorsal wall immediately behind the orifice of the common oviduct. This pouch (*sacculus*, figs. 1, 2, and 3, *p*) is lined by a very thick laminated cuticle with a projecting median ridge which appears to divide it into two lateral pockets. Each of these pockets opens behind into the uterus, and is usually filled with a clear colloid mass, which stains very deeply with alkaline carmine. It has all the appearance of being the same material as that which cements the eggs together when they have been deposited. The same contraction of the uterus which expels the egg would certainly expel some of this material from the uterine pouch.

4. *The Structure of the Gum-glands.*

Although I have used the term gum-glands to designate these organs, it will be seen that there is nothing in their structure to justify its use. And although they are usually regarded as secreting-glands which form a glue or cement for the attachment of the eggs, a function first apparently ascribed to them by Burmeister (7) and afterwards by Loew (18), Stein, who has examined these structures with more care perhaps than any other writer, entirely discards the view. He regards the so-called gum-glands as accessory organs of fertilization except in the Hydrophilidæ, where they open into the calyx of the ovary; and, curiously enough, disregarding the extreme improbability that gum-glands would open in such a situation, makes an exception in these insects, and regards the glands as gum-glands. Stein further identifies these glands in the Diptera with his "glandular portion of the organs of fertilization."

With regard to the histology of these glands, very little, if anything, can be said to have been recorded of a satisfactory character. Stein gives several very remarkable figures (*l. c.* pl. ix. figs. i, v, and xii) of their histological structure, with the following description:—

"The fine structure of the glands is nearly the same in all Beetles; they belong to that class of glands which yield a fluid secretion, and which are tubular, follicular, or exhibit bladder-like cavities. In the gland-follicles the proper elements of the gland form a manifold layer of nucleated cells which prepare the secretion. Between these cells very fine wavy canals spread into the follicles, formed as outgrowths of the epithelial coat (of the central cavity), and terminate either in blind ends or within the cells*.

"In general the contour of the gland-follicles is the same as that of the epithelial coat of the central cavity . . . . . . *The secreting-cells lie between the epithelial and peritoneal coats* [the italics are mine] without order, near and over each other, and not united together. In form they are round, oval, or egg-shaped; in the latter case the blunt end is turned outwards, and the outlines of the cells, when one examines the entire follicle under a certain pressure, are not generally distinctly seen, so many lie over each other, and the cells, owing to their granular contents, are so opaque" (pp. 102, 103).

Leuckart (16*a*), in his memoir on the Pupiparæ, gives a figure of the corresponding gland of *Melophagus ovinus*, which, although on a much smaller scale, represents a similar appearance, and gives a description which corresponds nearly with Stein's.

These figures and descriptions are very difficult to understand, except on the supposition that both Stein and Leuckart examined glands with a quantity of adherent fat-cells. The fat-cells of the ovary form a large mass on its posterior aspect, and closely surround and adhere to the gum-glands. These fat-cells, when half empty, as they always are in the egg-laying female, exhibit

* "Zwischen diesen Zellen verbreiten sich am Follikel sehr feine geschlängelte Kanäle welche von Austulpungen der Epithelealhaut gebildet werden, und die nach aussen entweder blind endigen, oder an einer Zelle endigen."

appearances, in optical section, which could be interpreted without difficulty, as Stein and Leuckart have interpreted them; possibly the fine tubes are the fine tracheæ of the fat-glands, whilst the cells figured by Stein are undoubtedly those of the fat-body, of which I give a figure (Pl. XXVIII. fig. 15) for comparison. Sir John Lubbock (19), describing the corresponding glands in *Coccus Persicæ*, gives a totally different description. He says:—

"They are six in number, four large and two small, the latter being apparently attached by a short stalk to the peduncle of the large one which is furthest from the vulva. They lie three on each side, and their ducts open into the egg-canal close together and about halfway between the vulva and the division of the egg-canal into two oviducts. The internal structure is very distinct and interesting. It consists of many cells lying loose in the internal cavity, and resembling very much in form, size, and appearance the vitelligenous cells of the egg-follicle."

I have been unable to find any other published details on the structure of these glands, which I shall now give from my own observations.

The gum-glands in the Blowfly are simple tubes, tortuous rather than convoluted, 2 m. in length, with an average diameter of ·175 m. They have a glistening white appearance, and are beaded over the surface from the projection of the cells lining them. In sections these glands are seen to consist of an outer musculo-cellular coat, like the so-called peritoneal coat of the oviduct. This is lined by a single layer of large epithelial cells. The lumen is filled by a granular fluid or semifluid substance. This is coagulated by alcohol, the granules suspended in it are blackened by osmic acid, and the intergranular material is scarcely stained by alkaline solutions of carmine. In this respect it differs entirely from the substance contained in the uterine pockets and from that with which the eggs are cemented together.

The epithelial cells which surround the lumen of the gland are irregular in form and measure, on an average, 80 μ in diameter, and from 30 to 40 μ in thickness. Many of these cells contain very remarkable spherical corpuscles, usually one in each cell (figs. 15 to 20). Besides these, some of the cells exhibit an oblong nucleus surrounded by a clear area (Pl. XXVIII. fig. 16).

In females in which the ovary is still without ripe ova (fig. 17) there are no corpuscles in the cells, but nuclei in an active state of division. Each nucleolus or each group of nucleoli, two or even four (figs. 17 and 18, *i*), is surrounded by a clear area. In some there is a small speck of deeply stained chromatin close to the nucleolus (fig. 19).

The epithelial cells (fig. 20, *e*) consist of distinctly reticular protoplasm and stain readily. The largest of the contained corpuscles measures 25 μ to 30 μ in diameter. A fully formed corpuscle exhibits a clear outer zone (fig. 20, *d*), 4 μ in breadth, with a distinct radial striation. This clear outer zone closely surrounds a finely granular contents (*c*) which stains feebly, and lying in it, usually near one side of the corpuscle, a clear vesicular spot (*b*) 5 μ in diameter, with a bright highly refringent spherule 2·5 μ in its centre (*a*).

These corpuscles have, in point of fact, the closest possible resemblance to the germ-ova of other animals.

The relation of these corpuscles to the nuclei of the containing cells must at present remain a matter of conjecture. It appears to me probable that one of the nuclei of the cells in the young gland, when there are two or more, develops the corpuscle whilst the others remain quiescent. The nuclei both of the young and mature cells stain deeply, whilst the vesicle and highly refractive body in the corpuscle remain unstained.

In several instances I have seen an appearance which leads me to believe that the corpuscles when mature are discharged from the cells in which they are developed into the lumen of the gland (fig. 20). Either empty spaces in the cells or a distinct fissure surrounding the corpuscle which lies close to the lumen of the gland are not uncommon. In some preparations the corpuscles, or some of them, have evidently fallen out in mounting the specimen.

On other occasions I have seen what appears to be a rupture of the clear external zone, and a protrusion of the contents of the corpuscle into the lumen of the gland. There is certainly a close similarity between the contents of these corpuscles and the material which fills the lumen of the gland.

I have, however, been unable to find either the corpuscles themselves or the vesicular body they contain imbedded in this material; but bright refractive nuclear particles like the central highly refringent body of the corpuscles undoubtedly exist in it.

5. *Theoretical Considerations and Conclusions.*

I am led by my observations to the following unexpected conclusions:—

The ovarian eggs in the Blowfly, and probably in other insects, are yelks, and contain no germ.

The so-called gum-glands are in reality germ-glands in which the germ-ova are developed.

These germ-ova pass into the yelks during their passage through the oviducts either (*a*) as naked germinal vesicles, or (*b*) as female pronuclei.

I shall now examine these hypotheses in relation to the work of previous investigators, and discuss their probability.

1. I have already shown that much difficulty exists in attempting to reconcile the observations of previous writers on the development of the ova in insects. So recently as 1881 Prof. Balfour (1) regarded the whole question as unsettled, and contented himself with stating that the relation of the ovum to the germogen and the relation of the yelk-cells to the ovum are points which have been especially controverted. I make this observation to show that the great number of researches which have been recorded by no means settle the question, which therefore still remains an open one.

2. The existence of true germ-ova, if such they are, in what has always been regarded as an accessory gland, although unexpected, is not inconsistent with the probable genetic relations of the Insecta.

Recent embryological observations show that the Insecta exhibit resemblances, sufficiently startling, to the Nemertid worms, and to the Trematodes generally, rather than to the Nematoid worms. This is seen by a comparison of the early developmental stages of *Lineus* (Barrois (2)) and *Chætognatha* (Kowalevski) with those of *Musca* (Kowalevski, Bütschli), my own unpublished observations being in complete accord with those of the above-named authors.

3. With regard to the structure of the so-called gum-glands of *Musca* and probably of other insects, I would submit that a comparison of the description of the gum-glands, as I have given it, with the ovary of some Arachnids, Crustacea, and Worms is not without interest.

That such ova are developed within cells and present appearances exactly similar to those I have described, is pretty evident from the figures of the young ovary of Spiders given by Stuhlmann (25), plate ix. figs. 190 and 197, and plate x. figs. 214, 215, and 216; whilst similar appearances are represented by Van Beneden (3, 4, and 5) in the germogen of the solid-bodied Worms and some Crustaceans. It is true these authors put a different interpretation on the relation of the ova to the epithelium of the germogen; but the fact remains that their figures are such as to show the close resemblance of the germogen in these animals and the so-called gum-glands of the fly.

4. With regard to the morphology of the gum-gland, so long as we remain ignorant of the precise manner in which this structure and the common oviduct are developed, its morphology will remain more or less doubtful. I have already given my reasons for the belief that the common oviduct is part of the primitive ovary; and this opinion is generally held. The condition of the parts in the Hydrophilidæ is an undoubted indication, I think, that the gum-glands are merely modified ovarian tubules, and have a similar origin from the primitive ovary; the connexion which persists between these glands and the calyx of the ovary in the fly is not, I think, unimportant in this relation. In the Hydrophilidæ the gland is comparable with the germ-gland of the Crustacea. Compare the figures given by Van Beneden (5) and by Stein (24).

It is true that Palmén (21) states that the appendicular glands (the gum-glands and the spermatophorous capsules) have the same origin from the ectodermal invagination as the vagina and uterus; but his observations are general, and he believed the gum-glands to open into the uterus.

5. Supposing my corpuscle to be a germ-ovum, its discharge from the cell in which it is developed may be considered an un-

paralleled phenomenon. I am by no means sure that it is so. The changes in the germarium of the Trematodes described by Van Beneden (5) appear to me to indicate a similar condition. The nature of the germ-ova in these has been discussed with great heat, some holding that the germ is a naked nucleus, and others that it retains a thin, scarcely demonstrable, layer of protoplasm around it.

6. Until the actual passage of such a germ into the yelk has been repeatedly observed, I admit that a serious hiatus exists in my hypothesis. I am far from sure that the phenomenon has not been already observed; at any rate a passage in Leydig's monograph (17) on the ovaries and seminal pouches of insects is worthy of note in this relation. Speaking of the chorion of *Musca domestica*, he says, "this has at the upper pole of the egg a prominent micropyle which appears blocked by a highly refractive corpuscle. The corpuscle is not present in all the eggs, and may be perhaps an exuding yelk-drop"*. I would ask, may it not equally have been an entering germ-yelk?

6. *Bibliography.*

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* "Dieselbe hat am oberen Eipol eine vorstehende Mikropyle und wie verstopft durch ein fettglänzendes Zäpfchen (Taf. iii. fig. 13*b*, 14*b*). Dieses Körperchen ist nicht bei allen Eiern vorhanden und entspricht vielleicht einem herausgetretenen Dottertröpfchen" (*l. c.* p. 35).

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DESCRIPTION OF PLATE XXVIII.

Figs. 1, 2, and 3. The uterus and its appendages in the adult egg-laying insect. The different parts are indicated by the same letters in all the figures.

b. Bursa copulatrix.
d. Duct of the gum-gland.
gl. Gum-gland.
m. Retractor muscle of oviduct.
o. Ovary.
od. Oviduct.
os. Terminal enlargement of the oviduct.
p. Uterine pouch.
s. Spermatophorous capsule.
ut. Uterus.
v. Vagina.
x. Attachment of the gum-gland to the ovary.

Fig. 1. Dorsal view of the uterus. × 20.

2. The same, with the ovaries. The parts rendered semitransparent with glycerine, and seen by transmitted light. × 10.

3. A median section of the uterus in the vertical antero-posterior plane.

4. The ovary of a three-day-old pupa. *c*, capsule; *cl*, cells of the calix; *es*, egg-strings. × 200.

5. Two of the egg-follicles from a ten-day-old pupa. *cl*, calicine duct; *os*, ovisac; *st*, stalk of egg-follicle of the ovary; *Psy*, pseudo-yelk-granules of the pupa; *x*, cell-mass to which the stalks of the ovisac are attached. × 300.

6. An ovisac from a young fly. *tc*, terminal chamber; *ec*, egg-chamber; *st*, stalk of ovarian follicle; *cl*, calicine duct; *tr*, trachea. × 200.

7. Another ovisac from a young fly, a little more advanced. *y*, edge of epithelium of egg; *ec'*, *ec''*, young egg-chambers. The other letters as in figs. 5 and 6. × 300.

Fig. 8. The egg-follicle from the ovary of a mature egg-laying insect. × 200.

9. Transverse section of an egg. *mc*, micropyle canal; *ch*, chorion; *v*, vitelline membrane; *cl*, clear yelk; *y*, granular yelk. × 30.

10. A longitudinal section of an egg. *mc'*, chamber at anterior pole of the egg; *m*, micropyle.

11. A section of the yelk. *a*, clear margin; *b*, granular yelk. × 400.

12. The micropyle. × 400.

13. A section through the micropyle. × 400.

14. The testes of a larval blowfly, showing the union of the prolongations from which the duct is developed: after Weismann.

15. The gum-gland and some of the adjacent fat-body. *l*, lumen of the gum-gland; *e*, epithelium of gum-gland; *bb*, capsule of fat-cells; *a*, *c*, *d*, stellate and flask-shaped cells enclosed within the capsule. × 200.

16. Transverse section of the gum-gland of the mature insect.

17. Transverse section of the gum-gland of the immature insect.

18, 19, 20. Epithelial cells from the gum-gland, with the contained corpuscles and nuclei in different stages of development.

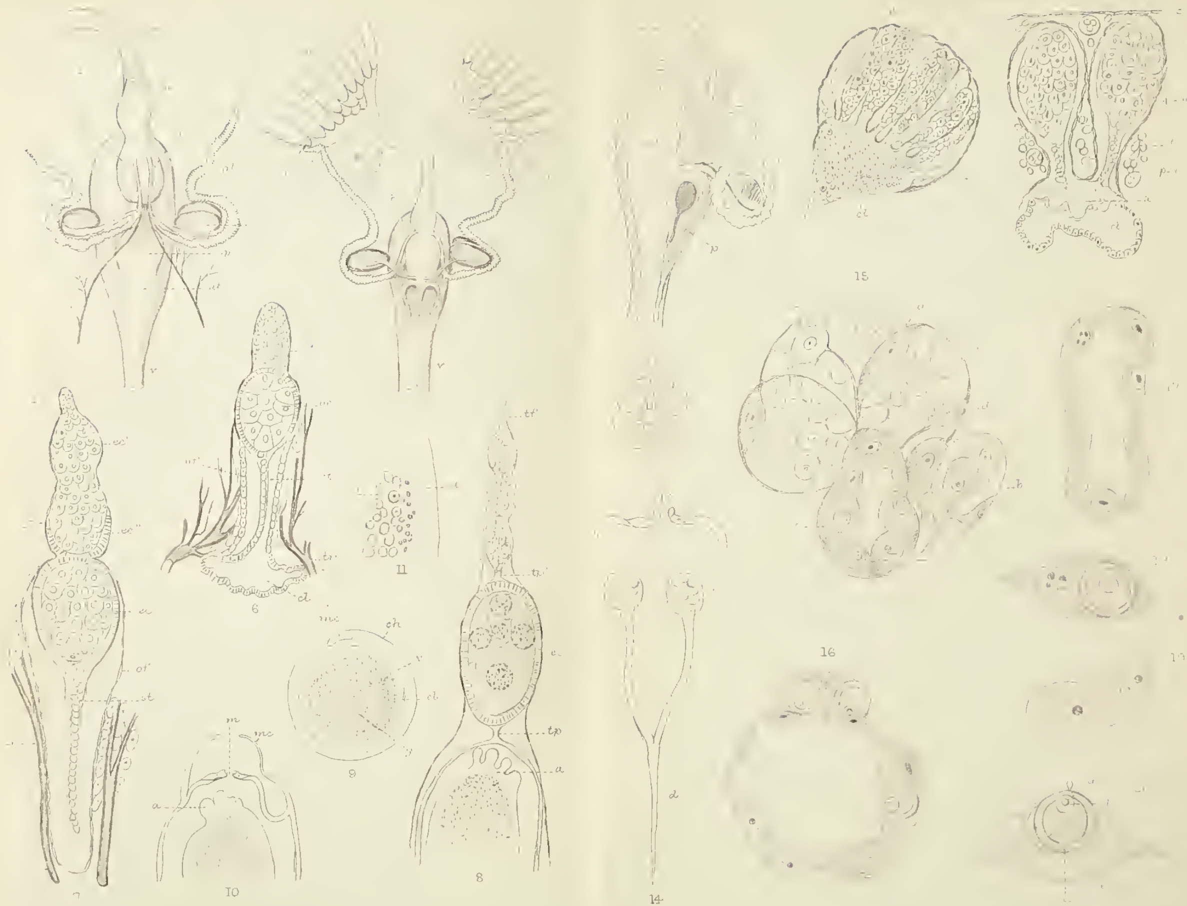
On the Deep-water Fauna of the Clyde Sea-area. By William E. Hoyle, M.A. (Oxon.), F.R.S.E., Keeper of the Manchester Museum. (Communicated by John Murray, LL.D., Ph.D., V.P.R.S.E., F.L.S.)

[Read 4th April, 1889.]

(With Map: Plate XXIX.)

Since the establishment of the Scottish Marine Station in the year 1884, Dr. John Murray has conducted an extensive series of dredgings in the greater number of the lochs of the west coast of Scotland. During these operations he was struck, as Forbes had been before him, with the restricted distribution of certain forms, as well as with the fact that some species occurred nowhere off the British shores except in these depressions.

In the summer of last year, Dr. Murray suggested that I



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OVARIES OF BLOWFLY AND THEIR APPENDAGES